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Original

Immunohistochemical detection of cathepsin D, estrogen receptor and progesterone receptor in malignant phylloides tumor and fibroadenoma of the breast

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RESUMEN

Planteamiento: Investigamos la expresión de catepsina D, receptores estrogénicos (ER), receptores de progesterona (PR) en tumores filodes malignos y en fibroadenomas de mama. Material y Métodos: Se estudiaron 9 tumores filodes malignos de mama y 6 fibroadenomas. Los cortes fijados en formol e incluidos en parafina se tiñeron con anticuerpos monoclonales para catepsina D, ER y PR. Resultados: En el citoplasma de las células epiteliales benignas de 14 casos (9 tumores filodes malignos y 5 fibroadenomas) se apreció tinción granular de catepsina D. El componente estromal del tumor no presentó expresión para catepsina D. En todos los fibroadenomas y tumores filodes malignos los núcleos de las células epiteliales presentaban positividad para ER. Los núcleos de las células estromales presentaban positividad en 2 fibroadenomas y en un tumor filodes maligno. Los PR fueron positivos en las células epiteliales de 10 casos (3 fibroadenomas y 7 tumores filodes malignos) y en las células estromales de 11 casos (3 fibroadenomas y 8 tumores filodes malignos). Conclusiones: La diferente expresión de la catepsina D en el componente estromal y epitelial indica diferencias en el estado de los receptores esteroideos. La expresión de la catepsina D en los elementos glandulares tumorales indica integridad en la vía de respuesta estrogénica. **Rev Esp Patol 1997; 30(3): 206-210.**

Palabras clave: Catepsina D - Receptor estrogénico - Receptor de progesterona - Tumor filodes maligno - Fibroadenoma - Mama

SUMMARY

Background: The authors studied the expression of cathepsin D (CD), estrogen receptor (ER) and progesterone receptor (PR) in malignant phylloides tumor and fibroadenoma of the breast. Material and Methods: Nine surgical specimens of malignant phylloides tumor and six fibroadenomas of the breast were included in this retrospective study. Sections from formalin-fixed, paraffin-embedded tissues were stained with CD, ER and PR monoclonal antibodies. Results: Granular CD staining of epithelial cytoplasm cells of benign glands was detected in 14 cases (9 malignant phylloides tumors and 5 fibroadenomas). Expression of CD was not found in stromal tumor component. Nuclear positive ER was found in epithelial cells of all cases of fibroadenomas and malignant phylloides tumors. Stromal cells of 2 fibroadenomas and one malignant phylloides tumor expressed nuclear ER. PR was found in epithelial cells in 10 cases (3 fibroadenomas and 7 malignant phylloides tumors) and stromal cells to 11 cases (3 fibroadenomas and 8 malignant phylloides tumors). Conclusions: Different expression for CD in both components may be due to differences in their steroid hormone receptor status. The results suggest that cathepsin D expression in glandular elements of the tumor reflects the functional integrity of the estrogen response pathway. **Rev Esp Patol 1997; 30(3): 206-210.**

Key words: Cathepsin D - Estrogen receptor - Progesterone receptor - Malignant phylloides tumor - Fibroadenoma - Breast

INTRODUCTION

Cathepsin D (CD) is a ubiquitously expressed lysosomal protease. Initially synthesized as an inactive precursor of 52 kd (pro-cathepsin D), the enzyme is subsequently converted to its active forms by proteolytic processing. This protease is induced by estrogens and growth factors (1, 2).

CD is expressed in the histiocytes (3). With the use of monoclonal antibodies levels of cathepsin D were very low or negligible in normal mammary glands (4).

Phylloides tumor is a relatively uncommon fibroepithelial tumor of the breast constituting 0.5% of all breast tumors (5). Microscopically the two key features of phylloides tumor are stromal hypercellularity and presence of benign glandular elements as an integral component of the neoplasm (6). Malignant phylloides tumors have marked nuclear atypia, numerous mitoses (generally exceeding 5 mitoses per 10 high-power fields), and loss of the relationship between glands and stroma (7).

Fibroadenoma is a common benign breast lesion. The stroma is usually made up of loose connective tissue. Occasionally, the cellularity of the stroma should be increased; in this case the alternative diagnosis of phylloides tumor should be considered.

In this report we have used monoclonal antibodies to CD, ER and PR to study the immunohistochemical expression in 9 cases of malignant phylloides tumor and six fibroadenomas of the breast.

MATERIALS AND METHODS

This study comprised 9 cases of malignant phylloides tumors of the breast obtained from the files of the Department of Pathology, Hospital del Bierzo, between 1985 and 1995. In all cases the size of tumor, number of mitoses of stromal component, necrosis, and expression of CD, ER and PR were evaluated.

All tissue specimens had been fixed in 10% formalin and embedded in paraffin wax. Sections were cut at 3-4 μm and mounted on poly-L-lysine-coated slides. For detection of CD, estrogen receptor (ER) and progesterone receptor (PR) the three-step steptavidin-biotin immunoperoxidase and the monoclonal antibody NCL-CDm (clon C5, Novocastra, Newcastle, England), ER (clon 1D5, Dako, Denmark), and PR (clon 1A6, Biogenex, California) were used. NCL-CDm was diluted 1:100, ER and PR were diluted to 1:50. Antigen retrieval for ER and PR was used. Slides were heated for 15 min At 700W in a microwave oven in 10 mM citrate buffer (pH6). The incubation time was 17-18 h. Development was performed by the peroxidase-diaminobenzidine (DAB) method. Appropriate positive and negative controls were used. The number of malignant stromal cell components and benign epithelial tumor cells varied among the cases. Thus, the percentage of the positive cells was estimated by counting 500 tumor cells in 10 high-power fields and semiquantitatively scoring them as follows: (–) completely absent (negative 0%); (+) less than 25%; (++) between 25% and 75%; and (+++) more than 75%.

RESULTS

Clinical features

The age range in patients with malignant phylloides tumors was between 27 and 61 years, with an average of 47.6 years. All patients were without evidence of disease at a median follow-up of 51.1 months (range 22-95 months). Palpable lymph nodes and metastases at diagnosis were not found. None of the patients experienced local recurrences or metastases. The age range of fibroadenomas was from 27 to 40 years with an average of 31.5 years. A median follow-up was 55.3 months (range 33-80 months).

Pathologic features

Gross findings

The malignant phylloides tumors were described as poorly circumscribed and firm nodular tumors. The nipple was flattened in one case (case 2). The cut surface was solid, gray-white, and showed cleft-like spaces. Median tumor size was 5.1 cm (range 2.5-8 cm) (Table 1). Fibroadenomas were well circumscribed and firm. Median tumor size was 3.2 cm (range 1.5-4 cm) (Table 1).

Microscopic findings

Four malignant phylloides tumors had pushing edges, and five were at least focally infiltrative. The tumors were comprised of the two typical components. Benign glandular elements and stromal hypercellularity were present in all tumors. Nuclear atypia, mitotic rate of more than five per 10 high-power fields (HPF) of the stromal component and loss of relationship between glands and stroma was present in all tumors (Table 1). In one tumor (case 2) the neoplas-

Table 1. Clinicopathologic features and expression of cathepsin D in malignant phylloides tumor and fibroadenomas.

Case	Age (years)	Size of tumor (cm)	Mitotic activity (10 HPF)	Epithelial			Stromal			Follow-up (months)
				Cathepsin D	Progesterone receptor	Estrogen receptor	Cathepsin D	Progesterone receptor	Estrogen receptor	
Malignant phylloides tumor										
1	61	2.5	9	+++/D	++/D	+++/F	–	+++/F	–	37
2	60	8	6	++/D	+++/D	+++/D	–	+++/D	–	35
3	46	4.5	6	+++/D	++/D	+++/D	–	++/D	–	95
4	27	6	7	+++/F	++/F	++/F	–	+/F	–	49
5	37	4	7	++/D	–	+++/D	–	++/F	–	34
6	52	6	6	+++/D	++/F	++/D	–	++/D	–	44
7	49	7.3	6	++/F	–	+/F	–	–	+/F	62
8	47	5	7	+++/D	+++/D	+++/D	–	++/D	–	54
9	50	3	6	+++/D	+++/F	++/D	–	+++/D	–	50
Fibroadenomas										
1	30	3	0	–	–	+/F	–	–	–	75
2	25	4	0	–	++	++/F	–	++/D	+/F	62
3	40	2.5	0	+/F	–	+/F	–	+/F	–	33
4	32	3	2	++/F	+/D	++/D	–	+/F	+/F	45
5	35	2	0	+/F	+/F	+/F	–	–	–	37
6	27	1.5	0	+/F	–	+/F	–	–	–	80

HPF: high power-fields. D: diffuse. F: focal.

tic stromal component was pleomorphic; metaplastic bone was encountered. All fibroadenomas had pushing edges and were composed of the two typical components. In one tumor (case 3) the epithelial component presented apocrine metaplasia.

Immunohistochemical results

Immunohistochemical staining results with CD, ER and PR are detailed in Table 1. In all cases of malignant phylloides tumors, there was unequivocal diffuse cytoplasmic and granular staining for CD in epithelial glandular cells (Fig. 1). Also, numerous stromal macrophages were stained positively. The intensity of epithelial cell tumor staining was variable (Table 1). Epithelial cells of fibroadenoma expressed focal cytoplasmic staining for CD in 4 cases (Table 1). Expression of CD was not found in stromal tumoral component of phylloides tumor and fibroadenoma.

Nuclear positive ER was found in all cases of malignant phylloides tumors and fibroadenoma (Fig. 2). The intensity of epithelial cell staining was variable (Table 1). Focal and weak expression of ER was found in stromal cells of fibroadenomas. Stromal cells of malignant phylloides tumor expressed ER in one case (Table 1). Focal positive PR was found in epithelial cells of 3 fibroadenomas and 7 cases of malignant phylloides tumors. Stromal cells of fibroadenoma and malignant phylloides tumors expressed PR in 3 and 8 cases, respectively (Table 1).

DISCUSSION

Phylloides tumor, a better term than the traditional cystosarcoma phylloides, was first described by Johannes Müller in 1838 (8). Since then, it has been investigated in order to define prognostic factors. Clinicopathologic evaluation (9), immunohistochemistry (10) and flow cytometry (11) have been performed to evaluate different features of the tumor, but uniform conclusions have not been reached.

CD has a wide tissue distribution and, interestingly, it appears to be regulated by estrogens in breast cancer (12, 13).

M. García et al. (14) described normal glands collected from plastic surgery which were negatively stained for CD, but occasional glands of the breast fibroadenomas were positively stained for CD. In a fibroadenoma included in

this study, the expression of CD was focal. Staining for stromal component in fibroadenomas was not present.

A different expression for CD in epithelial and stromal cells of phylloides tumor and fibroadenoma may be reflected by the local difference of their steroid hormone receptor status. Therefore, the expression of CD in benign epithelium of malignant phylloides tumor is produced because the specialized mammary stroma can induce the epithelial growth (7). This epithelial proliferation, the same as fibroadenoma, expressed CD, perhaps because their function was increased. Our study suggested that epithelial expression of CD was regulated and induced by ER status. All cases of malignant phylloides tumor expressed cytoplasmic CD and nuclear ER. Expression of CD was found in 66% of fibroadenomas. PR were expressed in numerous cases (67%).

In contrast, the tumoral stroma has PR but not ER (15). This feature may help to explain that stromal tumor cells failed to react with CD, perhaps because this protease is induced by estrogens (16). In our cases, ER in stromal cells was found to be focal, weak and insignificant. Stromal cells of fibroadenoma and malignant phylloides tumor showed PR.

Figure 1. Epithelial glands of malignant phylloides tumor showing strongly CD expression. Inset: Expression of CD was not found in stromal tumoral elements.

Figure 2. Positive nuclear estrogen receptor in epithelial cells.

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